REMARKS

Status of the Claims

Claims 1-3, 5-8, 10, 12, 14-29, and 31-35 are pending in the present application. Claims 4 and 30 are presently canceled. Claims 9, 11, 13, and 36 were previously canceled. Claims 8 and 14-27 are withdrawn as directed to a non-elected invention. Claims 1, 5, 8, 10, 12, 28, 29, and 34 are amended. Claims 1, 28, and 29 are amended to specify "wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment." Support for this element is found, *e.g.*, in previously pending claim 4. Claims 1, 5, 8, 10, 12, 28, 29, and 34 are also amended to clarify that the fibronectin is "recombinant" fibronectin. Support for this element is found, *e.g.*, on page 18, line 2, in the originally filed application. The claims are amended without prejudice or disclaimer and Applicants reserve the right to claim the canceled subject matter in one or more divisional or continuation applications. No new matter is entered by way of these amendments.

Entry of the above-described amendments is respectfully requested. Applicants submit that the claim amendments do not introduce new issues. The amendments incorporate subject matter, which was previously considered by the Examiner. The amendment specifying that the expanded cytotoxic lymphocytes maintain activity longer than those expanded without fibronectin was incorporated into the independent claims from previously pending claim 4. The clarifying amendment, *i.e.*, "recombinant" fibronectin, is implied from, *e.g.*, previously pending claim 29. If the Examiner believes that the claim amendments introduce new issues, the Examiner is respectfully requested to enter the amendment since it places the application in better form for appeal by materially reducing or simplifying the issues.

Information Disclosure Statement

The Examiner states that one of the references cited in the Information Disclosure Statement filed on January 22, 2009, could not be located in the file. Applicants submit herewith for the consideration of the Examiner a copy of the reference, *i.e.*, JP-2720712 and an English translation of the abstract.

Provisional Rejections Under the Obviousness-Type Double Patenting Doctrine

Claims 1-7, 10, 12, and 28-35 remain provisionally rejected on the ground of non-statutory double patenting over claims 1, 8, 15-16, 30, 32, and 34 of co-pending U.S. Application No. 10/486,512 in view of Mizobata *et al.*, *British J. Cancer*, 1996, 74:1598-1604, ("Mizobata"), and Chen *et al.*, 1994, *J. Immunol.*, 153:3630-3638, ("Chen") or over claims 1-15, and 20-21 of co-pending U.S. Application No. 10/568,745 in view of Mizobata, *see* Office Action, pages 3-4.

Claims 4 and 30 are canceled. Accordingly, the rejection is moot in regard to these claims.

The Examiner is respectfully requested to hold the provisional rejections in abeyance until allowable subject matter is identified in the present application.

Issues Under 35 U.S.C. § 103(a), Obviousness

Claims 1-7, 10, 12, 28-30, and 33-35

Claims 1-7, 10, 12, 28-30, and 33-35 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Davis *et al.*, *J. Immunol.*, 1990, 145:785-793, ("Davis") as evidenced by *Immunobiology, the immune system in health and disease*, (3rd edition), Janeway, C.A., & Travers, P. (Eds), New York: Garland Publishing Inc., pages 2-4, ("Janeway"), and Cardarelli *et al.*, *Cellular Immunology*, 1991, 135:105-117, ("Cardarelli"), in view of U.S. Patent No. 5,198,423 to Taguchi *et al.*, ("Taguchi"), *see* Office Action, pages 4-5. Applicants respectfully traverse.

The Examiner states that Davis teaches a method comprising incubating PBMCs for 4 days, which results in the expansion of CD8+ cells. Janeway is cited to support that CD8+ cells are cytotoxic T cells. The Examiner further alleges that expanding the number of CD8+ cytotoxic lymphocytes would result in an increase in cytotoxic activity. In addition, the Examiner states that Cardarelli teaches culturing PBMCs with immobilized fibronectin to increase IL-2 receptor expression. Taguchi is cited for teaching a fibronectin fragment comprising SEQ ID NO: 12.

Claims 4 and 30 are canceled. Accordingly, the rejection is moot in regard to these claims.

The burden is on the Examiner to make a *prima facie* case of obviousness, which requires an objective analysis as set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966). In *KSR International v. Teleflex Inc.*, 82 USPQ2d 1385 (2007), the Court affirmed that this analysis includes the following factual inquires: (1) determining the scope and content of the prior art; (2) ascertaining the differences between the claimed invention and the prior art; and (3) resolving the level of ordinary skill in the pertinent art. The Examination Guidelines for Determining Obviousness Under 35 U.S.C. § 103 in view of the Supreme Court Decision in *KSR International Co. v. Teleflex Inc.* state that, having undertaken the factual inquires of *Graham*, a rejection under 35 U.S.C. § 103 may be supported by one or more of the following rationales: (1) combining prior art elements according to known methods to yield predictable results; (2) simple substitution of one known element for another to obtain predictable results; (3) use of a known technique to improve similar devices in the same way; (4) applying a known technique to a known device ready for improvement to yield predictable results; choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success; (5) variations that would have been predictable to one of ordinary skill the art; and (6) some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine the prior art reference teachings to arrive at the claimed invention. 72 Fed. Reg. 57526, at 57529 (October 10, 2007). Each of the above-noted rationales requires predictability in the art and/or a reasonable expectation of success, and the Examiner must consider objective evidence, which rebuts such predictability and reasonable expectation of success. This objective evidence or secondary considerations may include unexpected results and/or failure of others (*e.g.*, evidence teaching away from the currently claimed invention), evidence of commercial success, and long-felt but unsolved needs, as found in the specification as-filed or other source. *Id.* When considering obviousness of a combination of known elements, the operative question is "whether the improvement is more than the predictable use of prior art elements according to their established functions." *KSR* at 1396.

Independent claim 1, as amended, is directed to a method for expanding cytotoxic lymphocytes which comprises: culturing precursor cells, capable of differentiating to cytotoxic lymphocytes, wherein the precursor cells are selected from the group consisting of peripheral

blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells in the presence of at least one recombinant fibronectin fragment together with interleukin-2, wherein the recombinant fibronectin fragment is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19, wherein said culturing is performed for 2-15 days, wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment.

Independent claim 28, as amended, is directed to a method for increasing expression of an interleukin-2 receptor in cytotoxic lymphocytes, which comprises: culturing precursor cells, capable of differentiating to cytotoxic lymphocytes, wherein the precursor cells are selected from the group consisting of peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells in the presence of at least one recombinant fibronectin fragment together with interleukin-2, thereby increasing expression of interleukin-2 receptor in the cells, wherein the recombinant fibronectin fragment is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19, wherein said culturing is performed for 2-15 days, wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment.

Independent claim 29, as amended, is directed to a method for increasing the number of CD8-positive cells in cytotoxic lymphocytes, which comprises: culturing precursor cells, capable of differentiating to cytotoxic lymphocytes, wherein the precursor cells are selected from the group consisting of peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells in the presence of at least one recombinant fibronectin fragment together with interleukin-2, thereby increasing the number of CD8-positive cells in the cultured cells, wherein the recombinant fibronectin fragment is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19, wherein said culturing is performed for 2-15 days, wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment.

Davis describes a method for proliferating T cells using native fibronectin, *see* page 786 of Davis, at the section entitled "Reagents." Davis' T cells were obtained by previously isolating and purifying the T cells from PBMC cells and then further fractionating the T cell population, *see* page 786 of Davis, at the section entitled, "Preparation of human peripheral blood T cells." Cell proliferation was measured by determining the amount of ^{3}H-thymidine uptake upon DNA synthesis of the cells after incubating the T cells for 96 hours, *i.e.*, for four days, *see* pages 786-787 of Davis, under the section entitled "Cell culture and assay of lymphocyte DNA synthesis."

Cardarelli describes a method for proliferating T cells by culturing peripheral blood lymphocytes on a plate coated with an anti-CD3 antibody and native fibronectin, *see* page 107 of Cardarelli under the section entitled "Reagents and antibodies." T cell proliferation is measured by determining the amount of ^{3}H-thymidine uptake upon DNA synthesis of cells, *see* pages 107-108 of Cardarelli under the section entitled "Isolation of human peripheral blood leukocytes and proliferation assay." Cardarelli further teaches that, when anti-CD3 antibody and native fibronectin are used, alone, the proliferation rate is higher than when anti-CD3 antibody and native fibronectin are used in further combination with IL-2. That is, in the absence of IL-2, Cardarelli report an excellent synergistic effect between anti-CD3 antibody and native fibronectin in the absence of IL-2. Based upon these results, Cardarelli conducted subsequent experiments without IL-2, *see* page 114, lines 18-20 of Cardarelli.

As noted above, the Examiner cites Taguchi for describing a fibronectin fragment comprising SEQ ID NO: 12. Janeway is merely cited to provide a general description of cytotoxic T cells.

Applicants submit that none of the cited references, either alone or in combination, teach or suggest all of the elements of the instant claims. As noted above, the claimed methods specify a method of culturing precursor cells capable of differentiating into cytotoxic lymphocytes. In contrast, the cells described in Davis and Cardarelli are not precursor cells, but cells that were previously isolated and purified from PBMC, *see* page 787, of Davis. Accordingly, the cells described in Davis are completely different from the precursor cells described in the instant claims.

Further, none of the cited references, either alone or in combination, describe a method

wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment. Davis and Cardarelli do not measure the cytotoxic activity of cytotoxic lymphocytes. Instead, Davis and Cardelli evaluate the proliferation rate of T cells by measuring the amount of DNA synthesized. Accordingly, Davis and Cardarelli fail to teach or suggest that their methods result in an expanded population of cytotoxic lymphocytes, which maintains its activity longer than expanded cytotoxic lymphocytes in the absence of fibronectin.

Neither Janeway nor Taguchi remedy the deficiencies of Davis or Cardarelli. Janeway is merely cited for generally describing cytotoxic T cells. Taguchi is merely cited for describing a fibronectin fragment comprising SEQ ID NO: 12. Accordingly, none of the cited references teach or suggest all of the elements of the instantly claimed methods.

Applicants further submit that an ordinary artisan could not have combined the cited references and reasonably expected to achieve the instant invention. In particular, an ordinary artisan would not have replaced the native fibronectin fragments described in Davis and Cardarelli with the recombinant fibronectin fragment described in Taguchi. At the time of the invention, an ordinary artisan recognized that native fibronectin is a gigantic molecule, which includes multi-functional regions, *see, e.g.*, page 17, lines 16 to page 18, line 4 in the application as originally filed. An ordinary artisan would have, accordingly, recognized that a gigantic multi-functional protein may have effects on cell culture that could not have been duplicated with a non-native fibronectin. Therefore, Applicants submit that an ordinary artisan could not have been reasonably certain that the proliferative effects observed by Davis and Cardarelli using native fibronectin would also have been observed with the fibronectin comprising SEQ ID NO: 12, as described by Taguchi. An ordinary artisan could not have disregarded the multi-functionality of native fibronectin and substituted the native fibronectin with a recombinant fibronectin and reasonably expected to achieve the instant invention.

Applicants further submit that Cardarelli teaches away from the instant invention. As noted above, Cardarelli teaches an improved effect on cell proliferation when anti-CD3 antibodies are combined with fibronectin without IL-2. In contrast, the instant claims describe IL-2 in combination with recombinant fibronectin, which indicates that IL-2 is an essential

element of the claimed methods. Accordingly, Cardarelli would have led an ordinary artisan away from the use of IL-2 in methods for expanding cytotoxic lymphocytes to maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment, as described in the instant claims.

Based upon the foregoing, Applicants submit that the instant claims are not obvious over the cited references. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claims 31-32

Claims 31-32 are also rejected as allegedly obvious over Davis, Cardarelli and Taguchi in further view of Chen, *see* Office Action, pages 5-6. Specifically, the Examiner admits that Davis, Cardarelli and Taguchi do not teach or suggest transducing a foreign gene into the T cell. However, according to the Examiner, Chen remedies this deficiency.

As noted above, Davis, Cardarelli and Taguchi do not teach or suggest all of the elements of the instant claims. In particular, neither Davis, Cardarelli, nor Taguchi teach or suggest culturing precursor cells capable of differentiating into cytotoxic lymphocytes. In addition, none of these references, either alone or in combination, teach or suggest that the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment. Further, an ordinary artisan would not have combined Davis, Cardarelli and Taguchi and reasonably expected to achieve the instant invention. In addition, Cardarelli teaches away from the instant invention.

Chen does not remedy the deficiencies of Davis, Cardarelli and Taguchi. Chen is merely cited for describing the transduction of a foreign gene into a T cell. Based upon the foregoing, the claims are not obvious over the cited references. Accordingly, Applicants respectfully request withdrawal of the rejection.

CONCLUSION

In view of the above amendments and remarks, Applicants believe the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Reg. No. 46,046, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

By _____ #42,874

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